# Urinary Amino Acids of Non-human Primates

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(Plates I-III; Text-figures 1-4)

#### I. INTRODUCTION

MALL but measurable quantities of free amino acids are regularly excreted in the urine of all mammals. Individual patterns of urinary amino acid excretion appear to be genetically determined and related to phylogenetic position. The present paper is a comparative study of the urinary amino acid excretion patterns of non-human primates.

The urinary amino acid excretion of humans has been the subject of normative and clinical studies and of genetic research. Berry (1953), Stein (1953) and Eades & Pollock (1954) determined the amino acid concentrations of urine specimens from normal human individuals. Smith (1958) reported on the association of unusual urinary amino acid patterns with various human diseases. The extensive literature on the inheritance of human amino acid excretion patterns has been reviewed by Harris (1959).

Only two previously published papers pertain to the urinary amino acid excretion of nonhuman primates. Datta & Harris (1953) included one rhesus monkey in their survey of mammalian urinary amino acid patterns. Gartler, Firschein & Dobzhansky (1956) reported on the amino acid excretion of 48 apes-2 gibbons, 3 orang-utans, 37 chimpanzees and 6 gorillas.

## II. MATERIALS AND METHODS

The general procedure followed in the present study is as follows. Specimens of urine were collected from 112 of approximately 200 primates kept at the Chicago Zoological Park at Brookfield, Illinois. A sample of each specimen was desalted and subjected to two-way paper chromatography. The amino acids separated on each urinary chromatogram were converted into visible spots by reaction with a color reagent. The quantity of amino acid represented by each spot was measured by comparing its optical density with the optical density of spots produced by known quantities of the corresponding pure amino acid.

As shown in Table I, the 112 animals studied belong to 7 of the 10 living families of nonhuman primates. Sixteen belong to 3 families of the Suborder Prosimii; 96 belong to 4 families of the Suborder Anthropoidea. The species and sex of the individuals studied are given in Table II. The classification used throughout is that of Simpson (1945), except for the generic subdivision of the Callithricidae, which follows Hershkovitz (1958).

All but two of the urine specimens were obtained by confining animals individually in a metabolism cage. Specimens from 106 of the 112 animals were collected over a 24-hour period of confinement. Specimens from the other

TABLE I. NUMBERS OF GENERA OF PRIMATES STUDIED COMPARED WITH NUMBERS OF LIVING GENERA

lividuals tudied
7
2
7
16
27
8
51
10
96

# TABLE II. SPECIES AND SEX OF ANIMALS STUDIED

Species	Males	Females	Total	Common Name
Family Tupaiidae				
Urogale everetti	2	5	7	Tree shrew
Family Lemuridae				
Lemur fulvus	1	1	2	Lemur
Family Lorisidae				
Nycticebus coucang	2	2	4	Slow loris
Perodicticus potto	1	0	1	Potto
Galago crassicaudatus	1	1	2	Galago
Family Cebidae			_	
Cacajao rubicundus	0	2	2	Uakari Saki
Pithecia monacha Cebus albifrons	0 4	1	1 5	Capuchin
Cebus apella	3	1	4	Capacinii
Cebus capucinus	1	1	2	
Cebus nigrivittatus	1	0	1	
Saimiri sciureus	2	1	3	Squirrel monkey
Ateles belzebuth	0 1	3 1	3 2	Spider monkey
Lagothrix cana	0	1	1	Woolly monkey
Lagothrix infumata	1	Ō	1	
Lagothrix poppigii	2 <sup>1</sup>	0	2	
Family Callithricidae				
Callithrix jacchus	0	4	4	Marmoset
Saguinus leucopus	0	1	1	Tamarin
Saguinus nigricollis	1	1	2	
Saguinus oedipus	1	0	1	
Family Cercopithecidae				
Macaca irus	0	3 1	3 2	Macaque
Macaca maura Macaca mulatta	1 0	2	$\frac{2}{2}$	
Macaca nemestrina	ŏ	3	3	
Macaca nemestrina $\times$ M. silenus	1	0	1	
Macaca radiata	2	4	6	
Macaca silenus	1	0	1 2	Mangahay
Cercocebus torquatus Papio cynocephalus	1 7	1 1	8	Mangabey Baboon
Comopithecus hamadryas	1	Ō	1	Hamadryas baboon
Mandrillus leucophaeus	0	2	2	Drill
Mandrillus sphinx	1	1	2	Mandrill
Cercopithecus aethiops	1	2	3	Guenon
Cercopithecus diana Cercopithecus l'hoesti	0	1 2	1 2	
Cercopithecus mitis	0	1	1	
Cercopithecus neglectus	1	2	3	
Cercopithecus talapoin		0	1	
Erythrocebus patas		0	1	Patas monkey
Presbytis entellus Presbytis obscurus	2 1	1 1	3 2	Langur
Colobus polykomos	1	0	1	Guereza
Family Pongidae	2			
Hylobates lar	1	2	3	Gibbon
Pongo pygmaeus		1	2	Orang-utan
Pan troglodytes	1	3	4	Chimpanzee
Gorilla gorilla		0	_1	Gorilla
Total	51	61	112	

<sup>1</sup>One, unavailable for re-examination, may be L. infumata.

6 individuals—1 orang-utan, 4 chimpanzees and 1 gorilla—were collected from a single urinary discharge. To assess individual constancy of amino acid excretion, a second urine specimen was obtained from 12 of the 106 animals originally represented by 24-hour specimens. Eleven of the 12 subsequent specimens were 24-hour collections; the other was from a single urination. A third 24-hour specimen was obtained from 1 animal.

Following the collection of each specimen its volume was measured, and after thorough mixing a sample was taken for amino acid determination. Because it was necessary to store the samples for a time between collection and analysis, isopropyl alcohol was added routinely as a preservative. The amount added was such that the original volume of urine equaled 90% of the final volume. The samples were stored at 4°C. for an average of approximately 8 months. The effect of storage on the amino acids of human urine has been studied by Stein (1953). He detected no change resulting from storage except for an increase in glutamic acid concentration. Stein attributed this increase to the presence in urine of a labile conjugate of glutamic acid which during storage is converted to the free amino acid.

Before chromatography each of the stored samples was desalted in a Research Specialties Co. electrolytic desalter in order to improve chromatographic resolution (Smith, 1958). A measured quantity, ranging from 20 to 200  $\mu$ l., of each desalted sample was applied as a 7-10 mm. spot centered 2.5 cm. from one corner of a 23 × 28 cm. sheet of Whatman No. 1 filter paper. Five or six sheets spotted with urine were mounted on a chromatography rack together with 5 or 6 sheets spotted with various concentrations of a known mixture of commerciallyobtained amino acids. In each instance 11 sheets, including unknowns and standards, were chromatographed simultaneously on one rack. Two chromatograms were made of each urine sample, usually on separate racks.

The first chromatographic solvent, which was run the length of the sheet, was a mixture (30:10:10:1) of 2,6-lutidine (Eastman Practical, 95%), absolute ethanol, distilled water and diethylamine. The second solvent, run at right angles to the first, was a mixture (100:20: 0.6) of liquefied phenol (Mallinckrodt Gilt Label, 88%), distilled water and ammonia. As recommended by Block, Durrum & Zweig (1958), a beaker containing 1% sodium cyanide in water was placed in the tank during phenol development. Following phenol development the sheets were allowed to air-dry thoroughly. Color was developed by dipping the dried sheets in a 0.2% solution of ninhydrin in acetone and subsequently heating them in a water-saturated atmosphere for 15 minutes at 76°C. Results of the technique are illustrated by Pl. I, Fig. 1, which shows the typical resolution of a known mixture of amino acids.

Within 15 hours after ninhydrin treatment, maximum optical densities of the developed spots were determined by means of a Welch Densichron transmission densitometer. Quantitative estimates of urinary amino acid concentrations were derived by interpolation from logarithmically plotted curves for standards developed on the same rack as the urine samples. Concentration values reported in this paper are the average of duplicate determinations. The distribution of differences between pairs of determinations is shown in Table III.

	1	Cumulative frequency in percent											
Concentration Log Difference between Duplicate Determinations <sup>1</sup>	Aspartic	Glutamic	Serine	Glycine	Glutamine	Lysine	Taurine	Threonine	Alanine	Hydroxyproline	Average		
<0.1	44	61	57	47	62	50	60	55	66	68	57		
<0.2	70	90	82	76	80	67	69	75	93	78	78		
<0.3	85	97	89	84	85	77	77	84	97	85	86		
<0.4	94	100	94	93	88	84	85	89	99	88	91		
<0.5	95	100	94	94	91	86	89	92	100	92	93		
<1.0	100	100	100	100	99	97	94	98	100	97	99		
<1.5	100	100	100	100	100	100	100	100	100	100	100		

TABLE III. DISTRIBUTION OF DIFFERENCES BETWEEN DUPLICATE URINARY AMINO ACID DETERMINATONS

<sup>1</sup>Concentration unit =  $10^{-5}$  mmoles/ml.

## III. RESULTS

A total of 10 urinary amino acids were chromatographically identifiable in the specimens studied. Traces of unidentifiable ninhydrin-positive substances were detected in some specimens. The clearly identified amino acids are: glutamic acid, aspartic acid, serine, glycine, glutamine, lysine, taurine, threonine, alanine and hydroxyproline. Table IV gives urinary concentrations of these 10 amino acids in specimens from the 112 individuals included in the study. The body weight of each animal and the volume of its 24hour specimen are also given.

Concentrations shown in Table IV are expressed logarithmically based on a concentration unit of 10-5 mmoles of amino acid per ml. of urine. In the interpretation of differences in concentration, a difference of 1.0 between concentration logs shown for two specimens obviously represents a tenfold difference in arithmetically expressed concentration. As indicated in a footnote to Table IV, concentration logs of zero are assigned to amino acids absent from a particular specimen or present in concentrations insufficient to yield a detectable chromatographic spot with the volume of urine applied.

TABLE IV. AMING ACID CONCENTRATION OF URINE SPECIMENS FROM 112 NON-HUMAN PRIMATES

			; ()		Log o	of cor	icentr	ation	in 10	- <sup>5</sup> mn	noles/	'ml.1	
Individual	Species	Body wt. (kg.)	Vol. of 24-hr. Specimen (ml.)	Aspartic	Glutamic	Serine	Glycine	Glutamine	Lysine	Taurine	Threonine	Alanine	Hydroxy- proline
I           33           34           35           38           39           40           41           37           42           43           45           53           36           139           132           133           47           138           94           96	SpeciesØUrogale everetti	0.27 0.27 0.34 0.28 0.28 0.28 0.35 0.41 2.9 3.2 1.1 0.56 0.42 1.5 0.76 n.d. <sup>2</sup> 1.1 1.0 3.5 2.7 n.d. <sup>2</sup> 2.3 2.3	13 23 37 12 11 9.4 9.0 37 96 22 37 11 30 37	I.9           1.6           1.3           1.6           1.3           0           0.7           0.4           0.6           1.0           1.3           0.9           0.6           1.0           1.3           0.8           1.3           0.8           1.3           0.9           0           1.0	2.0 2.3 2.4 1.4 1.8 2.0 1.8 1.6 1.1 0.6 1.3 1.7 1.6 2.3 2.7 1.6 2.3 2.8 1.7 1.9 1.4	1.7 1.6 1.4 1.1 0.7 1.4 1.4 1.7 0.6 1.3 1.3 1.1 1.0 2.0 1.2 2.3 2.0 0 1.9 1.8 1.8	D         I           2.3         2.1           2.3         2.1           2.3         2.0           1.8         1.6           2.1         2.2           2.1         2.2           3.2         2.1           2.4         2.9           3.5         1.6           2.5         2.1           3.5         3.3           2.6         2.7           2.2         2.2	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	$\begin{array}{c} \mathbf{\dot{r}}\\ 2.0\\ 1.5\\ 1.1\\ 0\\ 1.1\\ 1.6\\ 1.4\\ 1.1\\ 0.7\\ 0.8\\ 1.3\\ 0\\ 0\\ 0\\ 1.0\\ 1.3\\ 1.2\\ 1.9\\ 0.6\\ 0.6\\ 1.4\\ 1.3\\ 1.3\\ 0.6$	H         3.3         2.9         1.7         3.0         2.3         3.3         3.4         1.0         0         3.0         0         2.8         2.9         2.6         2.3         2.4         1.9         0         0         2.3         0         2.3         0         2.3         0         2.3         0         2.3         2.3         3         3         3         <	F           1.8           1.6           1.5           0           1.5           0           1.5           0           1.5           0           1.5           0           1.5           0           1.5           0           1.5           0           1.5           0           1.5           0           1.5           0           1.5           0           1.5           0           1.5           0           1.3           2.1           0.6           2.2           1.3           1.2           1.3           1.2           1.3           1.4	2.4           2.0           1.9           2.2           1.3           1.2           1.0           1.5           1.1           1.6           1.3           1.4           0.9           1.9           2.2           1.0           1.5           1.1           2.0           1.5           1.1           2.1           2.0           1.5           1.1           2.1           2.0           1.8           1.4	H 0 0 1.7 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
104 106 109	C. albifrons <sup>5</sup>	3.2 2.9 1.1	91 67 165	1.5 1.4 1.0	1.7 1.9 1.8	1.8 1.8 1.1	1.3 2.5 1.9	1.7 1.9 0.9	1.5 0 0.8	0.8 2.1 0	0.9 1.3 0.8	1.3 1.7 1.3	1.5 1.7 0
158 64 67 93 108 95	(Indiv. No. 109) <sup>5</sup> , 12 Dec. 58. 9 C. apella	n.d. <sup>2</sup> 2.9 2.3 4.1 5.6 4.8	n.d. <sup>2</sup> 165 116 63 36 90	1.6 0.8 1.0 1.0 2.3 1.1	2.1 2.1 2.4 1.7 2.4 2.1	1.0 0.7 1.2 2.0 2.0 2.1	2.1 1.6 2.2 2.7 1.9 2.8	1.3 0 1.3 2.3 2.0 1.8	0.6 0.9 1.1 1.5 1.5 1.2	1.9 2.5 1.6 2.9 2.0 0.7	0.7 1.1 1.5 1.9 1.6 1.4	1.9 2.0 1.3 1.8 1.9 1.9 1.9	1.3 1.0 1.9 2.2 2.2 1.6
93 137 97 55 157 56 140 66 118	C. capucinus $\qquad \qquad \qquad$	1.6 3.2 0.58 n.d. <sup>2</sup> 0.86 0.42 5.9 7.7	90 116 86 23 9.5 20 9.0 105 225	$1.1 \\ 1.4 \\ 1.1 \\ 1.0 \\ 1.6 \\ 1.2 \\ 1.6 \\ 1.0 \\ 1.4$	2.1 2.3 1.8 2.0 1.7 1.7 2.6 1.8 1.8	2.1 2.2 1.9 1.7 0 1.6 1.0 1.2 0.2	2.8 2.7 1.9 2.0 2.2 2.7 2.8 2.8	1.8 2.1 1.5 1.8 2.0 1.7 0 1.4 0	1.2 1.0 1.5 1.5 0.7 1.3 1.4 0 0.7	1.8 2.5 0 1.3 2.7 1.9 2.4 2.5	1.4 1.9 1.6 1.5 1.3 0 1.5 1.3 0.5	1.9 2.4 1.6 1.5 1.6 1.4 2.4 1.3 1.7	1.0 2.0 1.6 1.5 1.0 2.1 3.1 1.6 2.3

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		g.)	-hr. (ml.)		Log	of cor	icentr	ation	in 10	- <sup>5</sup> mn	noles/	ml.1	
Individual	Species	Body wt. (kg.)	Vol. of 24-hr. Specimen (ml	Aspartic	Glutamic	Serine	Glycine	Glutamine	Lysine	Taurine	Threonine	Alanine	Hydroxy- proline
ip         iii           119         122           136         58           63         65           117         48           49         50           52         60           128         129           59         62           1135         103           105         83           84         100           101         75           77         92           99         68           69         70           71         72           91         163           98         88           102         114           142         144           145         147           156         148           149         121           116         147	Species $\checkmark$ A. belzebuth <sup>5</sup> $\bigcirc$ A. geoffroyi $\bigcirc$ A. geoffroyi $\bigcirc$ Lagothrix poppigii $\bigcirc$ Lagothrix poppigii $\bigcirc$ L. cana $\bigcirc$ L. infumata $\bigcirc$ C. jacchus $\bigcirc$ C. jacchus $\bigcirc$ C. jacchus $\bigcirc$ Saguinus leucopus $\bigcirc$ S. nigricollis $\bigcirc$ S. nigricollis $\bigcirc$ S. nigricollis $\bigcirc$ Macaca irus, 16 Sep. 58 $\bigcirc$ (Indiv. No. 62), 8 Oct. 58 $\bigcirc$ M. irus $\bigcirc$ M. maura $\bigcirc$ M. maura $\bigcirc$ M. maura $\bigcirc$ M. maura $\bigcirc$ M. nemestrina $\bigcirc$ M. nemestrina $\bigcirc$ M. radiata $\bigcirc$ Papio cynocephalus $\bigcirc$ P. cynocephal	$\begin{array}{c} & & \\ & 5.0 \\ 10.9 \\ & 3.6 \\ & 3.6 \\ & 5.8 \\ & 6.4 \\ & 6.1 \\ & 0.23 \\ & 0.23 \\ & 0.23 \\ & 0.24 \\ & 0.32 \\ & 0.23 \\ & 0.25 \\ & 0.44 \\ & 0.38 \\ & 0.36 \\ & 4.1 \\ & 3.8 \\ & 5.4 \\ & 6.1 \\ & 5.2 \\ & 4.5 \\ & 5.4 \\ & 6.8 \\ & 7.3 \\ & 7.7 \\ & 2.3 \\ & 10.4 \\ & 16.8 \\ & 12.2 \\ & 16.7 \\ & 15.9 \\ & 6.8 \\ & 8.1 \\ & n.d.^2 \\ & 13.6 \\ & 18.6 \end{array}$	98 193 147 147 110 65 42 34 2.9 4.0 16 27 19	adge           0.7           0.3           1.6           1.1           0.3           1.1           0.9           1.1           0.9           1.1           0.9           1.1           0.9           1.1           0.9           1.1           0.9           1.1           0.9           1.1           0.9           1.1           0.4           1.5           1.1           1.4           1.5           1.1           0.4           1.2           1.3           0.9           0.1           1.0           0.2           0.7           0.9           0.9           0.9           0.9           0.9           0.9           0.9           1.3           0.9           0.9           0.9           1.8	E         E           1.4         2.0           1.7         1.6           1.3         2.3           0.9         3.0           1.7         2.6           2.1         1.7           2.6         2.1           1.7         2.0           1.2         2.4           1.5         2.0           1.8         1.6           1.3         2.0           2.3         1.8           1.9         2.0           1.4         1.2           2.0         1.9           2.0         1.4           1.2         2.1           2.2         2.1           1.2         2.1           1.2         2.1           1.2         2.1           1.2         2.1           1.2         2.1           1.2         2.1           1.2         2.1           1.2         2.1           1.2         2.1           1.4         1.1           1.2         2.0           1.4         1.1           1.9         2.5	-E         -E           1.4         0.6           1.9         1.2           0.8         1.2           2.3         1.2           1.6         1.1           0.6         0           1.2         0.8           1.2         0.6           0         1.2           0.6         0           1.2         0           0.6         0           1.3         0           1.4         1.1           0.6         0.9           1.4         1.1           0.6         0.9           1.4         1.1           0.6         0.9           1.4         1.1           0.6         0.9           1.0         0.3           0.8         1.0           0.3         0.7           1.3         1.1           1.0         0.6           0.7         1.3           1.10         0.6           0.7         1.3	S.           2.3           2.1           2.4           3.0           2.5           3.3           1.5           2.1           2.4           2.9           2.6           2.9           2.6           2.9           2.6           2.9           2.6           2.9           2.6           2.9           2.6           2.9           2.6           2.9           2.6           2.9           2.6           2.9           2.6           2.9           2.6           2.9           2.6           2.7           2.3           2.0           1.8           2.7           2.3           2.3           2.3           2.3           2.3           2.3           2.3           2.3           2.4           2.8           2.8           2.4      <	Jajo           1.2           1.2           1.7           0           1.0           2.3           0           1.6           0.9           1.1           1.0           0.5           1.4           0.5           1.3           1.4           0.8           1.1           2.2           1.3           0.5           1.0           0.5           1.0           1.5           0.5           1.0           1.5           0.5           1.0           1.3           0           1.4           1.5           1.3           1.4           1.7           1.3           0.5	$\begin{array}{c} \cdot \bar{g}_{A} \\ 0 \\ 0.6 \\ 0.5 \\ 1.1 \\ 1.4 \\ 1.1 \\ 0 \\ 0.9 \\ 0.8 \\ 1.0 \\ 1.5 \\ 1.4 \\ 0.5 \\ 0 \\ 0.5 \\ 1.3 \\ 1.0 \\ 0.5 \\ 1.3 \\ 1.0 \\ 0.5 \\ 1.3 \\ 1.0 \\ 0.5 \\ 1.3 \\ 1.0 \\ 0.5 \\ 1.3 \\ 1.0 \\ 0.8 \\ 1.4 \\ 0 \\ 0 \\ 0.8 \\ 1.5 \\ 0 \\ 1.2 \\ 1.6 \\ 0.8 \\ 1.0 \\ 0.8 \\ 1.0 \\ 0.8 \\ 1.0 \\ 0.8 \\ 1.0 \\ 0.9 \\ 0.4 \\ 0.8 \\ 1.0 \\ 0.9 \\ 0.3 \\ 0.4 \\ 0.4 \\ 0.8 \\ 0.9 \\ 0.3 \\ 0.4 \\ 0.4 \\ 0.8 \\ 0.9 \\ 0.3 \\ 0.4 \\ 0.4 \\ 0.8 \\ 0.9 \\ 0.3 \\ 0.4 \\ 0.4 \\ 0.8 \\ 0.9 \\ 0.3 \\ 0.4 \\ 0.4 \\ 0.8 \\ 0.9 \\ 0.3 \\ 0.4 \\ 0.4 \\ 0.8 \\ 0.9 \\ 0.3 \\ 0.4 \\ 0.4 \\ 0.8 \\ 0.9 \\ 0.3 \\ 0.4 \\ 0.4 \\ 0.8 \\ 0.9 \\ 0.3 \\ 0.4 \\ 0.4 \\ 0.8 \\ 0.9 \\ 0.3 \\ 0.4 \\ 0.4 \\ 0.8 \\ 0.9 \\ 0.3 \\ 0.4 \\ 0.4 \\ 0.8 \\ 0.9 \\ 0.3 \\ 0.4 \\ 0.4 \\ 0.8 \\ 0.9 \\ 0.3 \\ 0.4 \\ 0.8 $	Image         2.1           1.9         0           1.9         0           1.9         0           1.9         0           1.9         0           1.9         0           1.9         0           1.1         0           0         0.7           0         0           1.4         0           0         0           1.3         0           0         0           0         0           0         0           0.7         2.1           2.1         2.1           1.4         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0           0         0      0 </td <td>a         a           1.0         0           0         1.6           1.1         0           0.2         2.0           1.1         0.9           2.0         1.1           0.9         2.0           1.1         0.9           1.3         1.6           0         0.7           0.7         0           1.6         1.7           0.8         0           0.7         0.5           1.2         1.0           0.9         0           0.7         0.5           1.2         1.0           0.9         0           0.7         0.5           1.2         1.0           0.9         0           0.7         0.5           1.2         1.0           0.9         0           0.7         0.5           1.2         1.0           0.2         0           0         0           0.1.0         1.0           0.8         0</td> <td>Left           1.00           1.11           1.7           1.5           1.7           1.5           1.7           1.00           1.9           0.8           1.3           1.11           1.00           1.7           1.8           1.2           1.5           1.3           1.4           1.11           0.9           0.9           1.5           1.5           1.5           1.6           1.7           1.8           1.2           0.9           0.7           0.9           1.5           1.00           1.5           1.00           1.2           0.9           0.9           0.9           1.5           1.00           1.2           1.5</td> <td><math display="block"> \begin{array}{c} \text{IJoud} \\ 1.9 \\ 1.9 \\ 1.9 \\ 2.0 \\ 1.5 \\ 1.1 \\ 1.9 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 1.5 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ </math></td>	a         a           1.0         0           0         1.6           1.1         0           0.2         2.0           1.1         0.9           2.0         1.1           0.9         2.0           1.1         0.9           1.3         1.6           0         0.7           0.7         0           1.6         1.7           0.8         0           0.7         0.5           1.2         1.0           0.9         0           0.7         0.5           1.2         1.0           0.9         0           0.7         0.5           1.2         1.0           0.9         0           0.7         0.5           1.2         1.0           0.9         0           0.7         0.5           1.2         1.0           0.2         0           0         0           0.1.0         1.0           0.8         0	Left           1.00           1.11           1.7           1.5           1.7           1.5           1.7           1.00           1.9           0.8           1.3           1.11           1.00           1.7           1.8           1.2           1.5           1.3           1.4           1.11           0.9           0.9           1.5           1.5           1.5           1.6           1.7           1.8           1.2           0.9           0.7           0.9           1.5           1.00           1.5           1.00           1.2           0.9           0.9           0.9           1.5           1.00           1.2           1.5	$ \begin{array}{c} \text{IJoud} \\ 1.9 \\ 1.9 \\ 1.9 \\ 2.0 \\ 1.5 \\ 1.1 \\ 1.9 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 1.5 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ $
166 110 111 78	M. leucophaeus       9         M. sphinx       9         M. sphinx       8         Cercopithecus aethiops       9	2.3 4.5 4.5 3.4	275 521 n.d. <sup>2</sup> 59	1.4 1.2 1.2 0.5	1.7 2.5 2.0 1.4	1.1 1.1 1.5 0	1.9 2.7 2.4 2.9	2.8 2.2 2.3 0.8	0.9 0 0 0.8	1.0 0 0 0.8	0.7 0.7 0 0.3	1.0 1.6 1.4 1.1	1.3 0 1.4 0
79 86	C. aethiops 9 C. aethiops 8	3.9 7.9	250 250	1.0 0.5	1.7 2.1	0.2 0	2.4 2.8	0.7 1.2	0.8 1.1	0 0	0 1.0	1.0 1.0	0 0

			. î		Log o	of cor	icentr	ation	in 10	- <sup>5</sup> mn	noles/	ml.1	
Individual	Species	Body wt. (kg.)	Vol. of 24-hr. Specimen (ml.)	Aspartic	Glutamic	Serine	Glycine	Glutamine	Lysine	Taurine	Threonine	Alanine	Hydroxy- proline
87 130 73 74 81 131 80 82 85 164 107 127 120 113 114 115 89 90	C. diana, 25 Sep. 58	2.7 2.7 2.3 4.3 4.3 n.d. <sup>2</sup> 6.6 3.9 4.3 5.4 1.4 n.d. <sup>2</sup> 13.6 11.7 10.4 15.4 15.4 15.4 1.7 10.4 2.7 2.7 2.7 2.7 2.7 2.7 2.7 2.7 2.7 2.3 4.3 4.3 1.3 5.4 1.4 1.4 1.4 1.4 1.4 1.4 2.5 2.4 1.4 1.4 1.4 1.4 1.4 1.4 1.4 1.4 1.4 1	193 202 280 190 400 250 275 175 s.u. <sup>6</sup> 76 92 86 188 468 (650) <sup>3</sup> 134 200	$1.4 \\ 2.0 \\ 1.1 \\ 1.2 \\ 0.7 \\ 1.1 \\ 1.0 \\ 1.3 \\ 0.4 \\ 0.8 \\ 1.1 \\ 1.1 \\ 1.4 \\ 0.7 \\ 0.9 \\ 0.6 \\ 1.2 \\ 1.5 $	3.0 2.8 1.2 1.5 1.1 1.2 2.6 1.9 2.7 2.4 1.4 1.5 1.5 1.8 1.5 1.8 1.5 1.8 1.4 2.5	0 1.3 0 0.3 0 0 0 0 0 0 0 0 0 0 0 0 0	2.8 2.9 2.7 2.8 3.0 3.5 2.7 2.5 2.5 2.0 2.2 2.3 2.0 2.8 2.5 3.1 2.6 2.7	2.2 2.8 1.1 1.7 1.2 2.2 1.2 0 1.8 0 1.0 1.3 0.6 2.4 0 2.0 2.7 2.7	$\begin{array}{c} 0.5\\ 0\\ 1.1\\ 0.4\\ 0\\ 0.7\\ 0.9\\ 0\\ 1.1\\ 1.0\\ 0.3\\ 0\\ 1.3\\ 1.4\\ 0\\ 1.2\\ 1.1\\ 0.9 \end{array}$	0 0 0 0.5 2.1 0.6 0 1.7 0 1.6 2.0 2.1 1.9 2.2 2.1	$\begin{array}{c} 1.3\\ 1.6\\ 0.5\\ 0\\ 0\\ 0.8\\ 0.4\\ 0\\ 1.5\\ 1.1\\ 0.3\\ 0\\ 1.2\\ 1.9\\ 0.7\\ 1.5\\ 2.1\\ 1.4 \end{array}$	2.3 2.3 1.2 1.5 1.1 1.5 1.9 2.0 2.2 1.3 1.3 0.8 1.2 1.0 1.3 1.4 1.7	0 1.1 0 0.6 0.8 0 0 1.4 0.5 0 0 0.5 0.6 0.5 1.2 0
54 126 150 151 152 123 153 159 160 161 162 154	$Colosultation8Colosultation8Colosultation812 Sep. 58.8(Indiv. No. 54), 20 Oct. 58.8Hylobates lar9H. lar9H. lar9Pongo pygmaeus9P. pygmaeus8Pan troglodytes9P. troglodytes9P. troglodytes9P. troglodytes9P. troglodytes9P. troglodytes9P. troglodytes9Gorilla gorilla^78$	13.6 n.d. <sup>2</sup> 4.5 6.4 5.0 15.9 24.9 12.7 14.5 15.9 10.4 n.d. <sup>2</sup>	425 283 50 172 153 n.d. <sup>2</sup> s.u. <sup>6</sup> s.u. <sup>6</sup> s.u. <sup>6</sup> s.u. <sup>6</sup> s.u. <sup>6</sup> s.u. <sup>6</sup>	0.4 0.7 1.0 0.9 0.8 1.1 0.5 0.5 0.5 0.3 0.5 1.2 1.0	$\begin{array}{c} 1.3 \\ 1.6 \\ 1.5 \\ 1.0 \\ 0.9 \\ 1.6 \\ 0.4 \\ 0.5 \\ 0.6 \\ 0.8 \\ 1.3 \\ 1.6 \end{array}$	0.3 0.6 1.4 1.2 1.1 0.4 0.6 0.3 0.3 0.4 1.0 0.9	2.4 1.9 2.0 2.3 1.7 2.4 1.5 1.3 1.4 1.6 1.5 2.2	$\begin{array}{c} 0.3 \\ 1.1 \\ 1.2 \\ 1.3 \\ 1.2 \\ 0.9 \\ 0.1 \\ 0.9 \\ 1.3 \\ 0 \\ 1.3 \\ 1.4 \end{array}$	0 0.9 1.4 0.9 0.6 0.3 0.6 0 0.1 0.2 0.9 0.8	0 0.8 1.3 0 1.0 1.2 1.2 2.0 1.5 2.1 2.2 1.0	0 0.9 0.8 0 0.6 0.5 0.3 0.1 0.8 0.2 0.9 0.8	0.9 0.7 1.6 1.3 0.9 1.5 0.7 0.1 -0.1 0.7 0.9 1.0	0 0.7 0 0 0.9 0 0.9 0 0.6 0.1 0 0

TABLE IV. AMINO ACID CONCENTRATION OF URINE SPECIMENS FROM 112 NON-HUMAN PRIMATES (cont.)

<sup>1</sup>Zero values are assigned to amino acids whose concentration was insufficient to yield a detectable chromatographic spot with the volume of urine applied.

<sup>2</sup>Not determined. <sup>3</sup>Approximate volume.

<sup>4</sup>Specimen from this species is composite collection of 24-hour output of two female uakaris.

<sup>5</sup>Cebid individual fed meatless diet.

<sup>6</sup>Specimen represents single urinary discharge.

<sup>7</sup>Specimen from this animal siphoned off previously scrubbed floor of exhibition cage.

#### IV. DISCUSSION

## A. Urinary Output and Body Weight

In Text-fig. 1 generic mean logs of 24-hour urinary output in ml.  $(U_{24 \text{ hr}})$  are plotted against corresponding mean logs of body weight in grams (B). Superimposed on these points is the line

$$U_{24 \text{ hr.}} = 0.1536 \text{ B}^{0.82},$$

which is equivalent to

 $U_{1 hr.} = 0.0064 B^{0.82},$ 

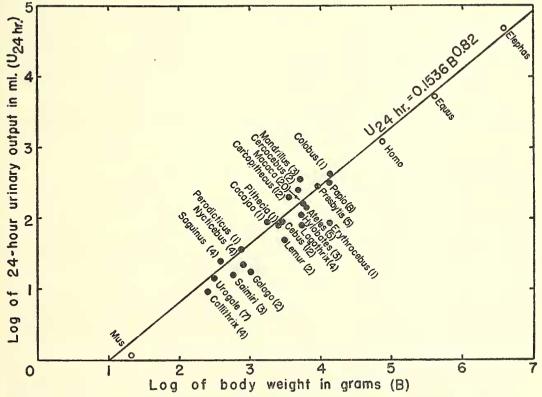
the equation found by Adolph (1943; 1949) to express the constant relationship between urinary output and body weight in mammals of diverse orders. Also shown on the graph are values from Adolph for man and for three non-primate genera. The primate data apparently conform to the general mammalian relationship. This casts doubt on the assumption of Gartler, Firschein & Dobzhansky (1956) that the low urinary creatinine concentration of gibbons, orangutans, chimpanzees and gorillas is compensated for by a high daily volume of urine per unit of body weight.

B. Successive Specimens from Individuals The amino acid concentrations of urine specimens from the 12 individuals sampled more than once are compared graphically in Text-fig. 2. These graphs show the successive specimens of each individual to be generally similar in urinary amino acid pattern. Taurine concentrations, however, do vary considerably in many cases, for 5 of the 12 re-sampled individuals taurine concentration log differences between successive specimens are 1.0 or greater. The general constancy of amino acid pattern in successive specimens from these non-human primates is in accord with the constant urinary amino acid patterns reported for human individuals by Berry (1953) and Harris (1953). Plates I and II, Figs. 2-4, are photographs of chromatograms comparing the successive specimens of 3 resampled individuals.

## C. Effect of Diet

The results of previous studies (Sutton, 1951; Gartler, Firschein & Dobzhansky, 1956; Smith, 1958) indicate that the urinary amino acid excretion of humans is generally unaffected by normal variations in diet. In the course of the present study an opportunity to observe the effect of certain dietary factors on the urinary

amino acid excretion of non-human primates was afforded by the housing and feeding arrangement at the Brookfield Zoo. Prosimians and most South American monkeys are kept in the Small Mammal House, and old world monkeys are kept in the Primate Building. Primates in the Small Mammal House are fed fruit, vegetables, bread and raw ground horsemeat fortified with a vitamin-mineral supplement. The basic diet in the Primate Building includes only fruit, vegetables and bread. Meat is not given in the Primate Building, although animals of the following species do receive a raw egg daily: Cebus albifrons, Cebus apella, Macaca nemestrina X M. silenus, Macaca silenus, Cercocebus torquatus, Comopithecus hamadryas, Mandrillus leucophaeus, Mandrillus sphinx, Cercopithecus diana, Erythrocebus patas. The phylogenetic separation of housing facilities outlined above is not maintained completely. Of the South American monkeys, 4 of 12 capuchins (Cebus) and 3 of 5 spider monkeys (Ateles) are kept in the Primate Building. The 7 cebids housed in the Primate Building receive a diet identical with that of the old world monkeys; these animals are individually identified in Table IV.



TEXT-FIG. 1. Urinary output in relation to body weight. Closed circles=means for primate genera studied (number of specimens in parentheses); open circles=values for man and non-primates, from Adolph (1943). Superimposed straight line also from Adolph.

In Table V the capuchins and spider monkeys fed meat and those not fed meat are compared with respect to their frequency distributions of urinary amino acid concentrations. The two groups differ consistently only in distribution of threonine concentrations. The feeding of fortified meat thus appears to have no general effect on the urinary amino acid excretion patterns of these cebids. A similar comparison of urinary amino acid concentrations of species fed eggs and of those not fed eggs reveals that this dietary factor also is without apparent effect on urinary amino acid excretion.

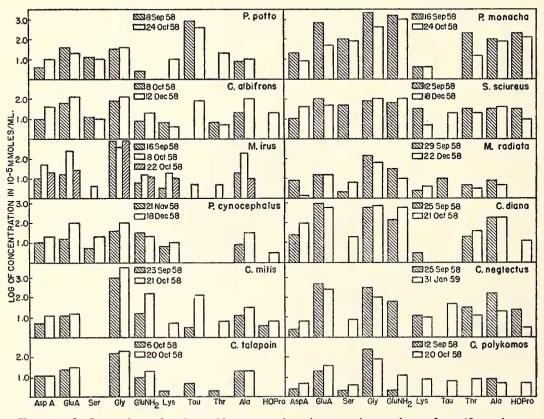
## D. Phylogenetic Comparisons

Examination of Table IV reveals no consistent differences in concentration between species within a given genus. Accordingly, for purposes of phylogenetic analysis, attention is centered on comparison of the urinary amino acid patterns of genera and families.

Generic means and standard errors of urinary amino acid concentrations are represented graphically in Text-fig. 3. These means are based on data in Table IV for the 106 animals represented by 24-hour specimens. As there appear to be no sexual differences in urinary amino acid concentrations, data for both males and females are included in the computation of each generic mean. Data for the 6 animals represented only by specimens passed at a single urination are not included; the concentration level of these specimens may differ from that of the 24-hour specimens. Individuals from which two or more specimens were collected are represented by average concentrations.

Inspection of Text-fig. 3 reveals that the 10 amino acids identified differ considerably in inter-generic uniformity of concentration. Aspartic acid, glutamic acid, glycine and alanine maintain relatively constant concentrations in the urine of the 24 genera studied. Inter-generic variations of mean concentration of these 4 amino acids are small relative to intra-generic variations, represented by the standard errors. The other 6 amino acids show a relatively greater degree of inter-generic variation of mean concentration.

For the 4 amino acids of relatively constant concentration in primate urine, over-all concen-



TEXT-FIG. 2. Comparison of amino acid concentrations in successive specimens from 12 non-human primate individuals.

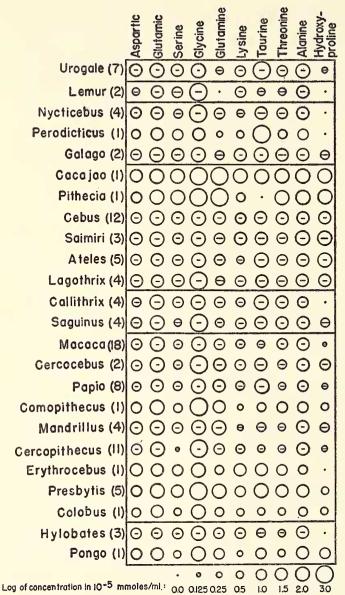
	Log of amino acid concentration in 10-5 mmoles/ml.											
Diet	<0.5	0.5 to 0.9	1.0 to 1.4	1.5 to 1.9	2.0 to 2.4	2.5 to 2.9	<0.5	0.5 to 0.9	1.0 to 1.4	1.5 to 1.9	2.0 to 2.4	2.5 to 2.9
			С	ebus	·	•		A	teles			
					Aspa	rtic Acio	1					
Meat Meatless	1	1	6 2	1	i	•	1	1	1 1	1	•	•
Meaness	Glutamic Acid									•		
Meat			1	3	4		u			2	•	
Meatless	•	•	•	3 2	2		•	•	1	1	1	
Serine												
Meat	•	1	1 1	3 2	3 1	•	i	i	1 1	1	•	•
Meatless	•	•	1	2	-	lycine	1	1	1	•	•	•
Meat				1	2	s 5					1	1
Meatless			1	1	1	1	•				2	1
Glutamine												
Meat	1	•	1 1	3 2	3 1	•	i	•	1 2	1	•	•
Meatless	•	•	I	2		•	1	•	2	·	•	•
Meat		1	5	2	Lj	ysine	1		1			
Meatless	1	1	•	2 2	•	•	•	3	•	•	•	•
					Ta	urine						
Meat	1	1	:	2	1	3	;	•	•	1	1	;
Meatless	•	1	1	·	2	•	1	•	•	•	1	1
Meat		·	2	6	Inr	reonine			1	1		
Meatless	•	2	1	1		-	i	1	1			
					Al	anine						
Meat		•	2 1	5 3	1	•		•	1 2	1	•	•
Meatless	•	•	1	3		•	•	•	2	1	•	•
Meat			1	4	Hydro 3	oxyproli	ie			2		
Meatless		i	1	2	1	•	•		•	2	i	:

TABLE V. EFFECT OF DIET ON URINARY AMINO ACID EXCRETION OF 17 CAPUCHINS AND SPIDER MONKEYS

tration means may be taken as approximations of values for the order as a whole. These over-all means are given in Table VI. Glycine, with an average concentration of 2.40 µmoles per ml., is the most prominent amino acid in primate urine. Glutamic acid has the next highest average concentration, 0.63  $\mu$ moles per ml., about onefourth the mean glycine concentration. It should be noted, however, that, because of the previously mentioned effect of storage, all of the glutamic acid measured may not originally have been present as the free amino acid. Urinary alanine and aspartic acid are much lower in mean concentration. The average alanine concentration is about one-tenth that of glycine; the average aspartic acid concentration, about onetwentieth.

The urinary glutamic acid and aspartic acid concentrations reported above for non-human primates are much higher than human urinary concentrations of these two amino acids. This agrees with one of the principal findings of Gartler, Firschein & Dobzhansky (1956), who found glutamic acid and aspartic acid concentrations in ape urine to be significantly higher than in human urine. The high urinary glycine concentrations found in the present study also agree with the results of Gartler, Firschein & Dobzhansky; they do not agree with the apparent absence of glycine from the urine of the single rhesus monkey studied by Datta & Harris (1953).

The 6 amino acids showing relatively great inter-generic variation of concentration—namely, serine, glutamine, lysine, taurine, threonine



and hydroxyproline—are those among which evidence is to be sought concerning phylogenetic trends in primate urinary amino acid excretion. Generic frequency distributions of concentrations of these 6 amino acids are compared graphically in Text-fig. 4, from which are omitted genera represented by only a single urine specimen. Generic excretion tendencies indicated by the frequency distributions are presented in Table VII. Phylogenetically these may be summarized as follows:

At the subordinal level, prosimian genera all tend to be low in urinary glutamine. At the family level, tupaiids, as represented by the single TEXT-FIG. 3. Comparison, by genera, of mean amino acid concentrations of urine specimens from 106 non-human primates. Area of each circle proportional to generic mean; bar within circle represents diameter of standard error of mean; horizontal lines separate data for each family.

species available for study, appear to be high in urinary serine, lysine and taurine. Cebids as a family are uniquely high in urinary hydroxyproline; among cebid genera, *Cebus*, represented by 12 individuals of 4 species, clearly tends to be high in all 6 generically variable urinary amino acids. The two callithricid genera studied tend slightly to be high in urinary taurine. Cercopithecids generally are low in the generically variable urinary amino acids other than glutamine; most extreme in this respect is the genus *Cercopithecus. Papio* and *Presbytis*, however, are exceptional; *Papio* is high in urinary taurine, and *Presbytis* is high in serine, lysine, taurine, and threonine.

P	ooden:	Urinary	Amino

Amino Acid	Mean and Standard error of Concentration Logs <sup>1</sup>	Mean Concentration in $\mu$ moles/ml
Aspartic acid	$1.05 \pm 0.04$	0.11
Glutamic acid	$1.80 \pm 0.04$	0.63
Glycine	$2.38\pm0.04$	2.40
Alanine	$1.41 \pm 0.04$	0.26

TABLE VI. MEAN CONCENTRATION OF ASPARTIC ACID, GLUTAMIC ACID, GLYCINE AND ALANINE IN URINE SPECIMENS FROM 106 NON-HUMAN PRIMATES

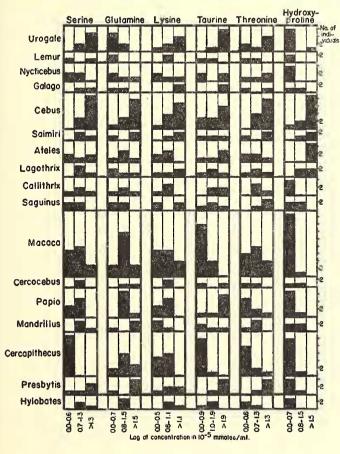
<sup>1</sup>Unit of concentration =  $10^{-5}$  mmoles/ml.

The hydroxyproline content of cebid urine is distinctive. Twenty-two of 26 cebid specimens have hydroxyproline concentration logs of 1.5 or greater; only 2 of 79 non-cebid specimens have hydroxyproline concentrations this high. The mean urinary hydroxyproline concentration log for cebids is  $1.83 \pm 0.11$  (0.66 µmoles per ml.); the corresponding mean for non-cebids is  $0.27 \pm 0.08$  (0.02 µmoles per ml.). The hydroxyproline spot characteristic of cebid urinary chromatograms is illustrated in Pl. III, Fig. 5, which shows photographs of one chromatogram from each of the 6 cebid genera studied. These

chromatograms also illustrate the dietary independence of cebid urinary hydroxyproline excretion. Although only the *Ateles* chromatogram is from an individual not fed meat, its hydroxyproline spot is not different from the others.

#### V. SUMMARY

Urinary amino acid excretion patterns have been studied chromatographically in specimens from 112 primate individuals representing 7 of 10 non-hominid families. Analysis of successive specimens from 12 animals indicates that urinary amino acid patterns of primate individuals



TEXT-FIG. 4. Distributions of urinary serine, glutamine, lysine, taurine, threonine and hydroxyproline concentrations in 16 non-human primate genera.

Suborder	Amino Acid Concentration Level <sup>1</sup> of Genus										
Family Genus	Serine	Glutamine	Lysine	Taurine	Threonine	Hydroxy- proline					
Prosimii											
Tupaiidae											
Ūrogale	+	0	+	+	(+)	0					
Lemuridae											
Lemur		0		(0)	(0)	0					
Lorisidae											
Nycticebus	0	(0)	(0)		(+)	0					
Galago	(+)	(0)	+	+		(0)					
Anthropoidea					و						
Cebidae											
Cebus	+	+	+	+	+	+					
Saimiri	(+)	(+)	+ + 0		(+)	+++++++++++++++++++++++++++++++++++++++					
Ateles				+	(0)	+					
Lagothrix	(+)	(0)	(+)			+					
Callithricidae											
Callithrix			(0)	+	(+)	0					
Saguinus	0	0			+	0					
Cercopithecidae											
Macaca	0		0	0	(0)	0					
Cercocebus		(+)	0		(0)	(0)					
Papio	0	(+)	0	+	0 (0)	0					
Mandrillus Cercopithecus	(+)	+	0	(0) 0	0	0					
Presbytis	0 +	(+) +	(+)	+	+	Ő					
Pongidae	Ŧ	4.	()	.1.	•	-					
Hylobates	(+)		(+)	(0)	0	0					
	(+)										

TABLE VII. URINARY AMINO ACID EXCRETION TENDENCIES OF PRIMATE GENERA

1 + = high; O = low; () = weak tendency.

remain relatively constant through time. Dietary variations appear in general to be without effect on urinary amino acid excretion. Daily urinary output bears a constant exponential relationship to body weight.

The primate genera studied tend to be uniform in urinary concentration of glycine, glutamic acid, alanine and aspartic acid. Average concentrations of these 4 amino acids, expressed in  $\mu$ moles per ml. of urine, are as follows: glycine, 2.40; glutamic acid 0.63; alanine, 0.26; aspartic acid, 0.11. Inter-generic variations of concentration are relatively great for glutamine, serine, lysine, taurine, threonine and hydroxyproline. Prosimians in general are low in urinary glutamine. Tupaiids, represented in the study by one species, appear to be high in urinary serine, lysine and taurine. Cebids are uniquely high in urinary hydroxyproline; the genus Cebus also is high in serine, glutamine, lysine, taurine and threonine. Cercopithecids tend to be low in inter-generically variable uriinary amino acids other than glutamine; Papio, however, is high in taurine, and *Presbytis* is high in serine, taurine and threonine.

## ACKNOWLEDGMENTS

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## **EXPLANATION OF THE PLATES**

## PLATE I

- FIG. 1. Typical chromatographic resolution of known mixture of amino acids.
- FIG. 2. Chromatographic comparison of amino acids in successive urine specimens collected on indicated dates from male *Perodicticus potto*.

## PLATE II

FIG. 3. Chromatographic comparison of amino acids in successive urine specimens col-

lected on indicated dates from female *Macaca irus*.

FIG. 4. Chromatographic comparison of amino acids in successive urine specimens collected on indicated dates from female *Cercopithecus neglectus*.

#### PLATE III

FIG. 5. Urinary chromatograms representing each of six cebid genera studied. Arrows indicate hydroxyproline spots.